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Research Article

MOLECULAR GENETIC VARIATION OF *RHYNOCORIS MARGINATUS* BASED ON MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT I GENE (HETEROPTERA : REDUVIIDAE)

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ABSTRACT

The Assassin bugs of the genus *Rhynocoris* are mostly predatory and biocontrole insect pest with currently close to 190 species descried in worldwide. In this present study was we investigate the genetic variation of mitochondrial gene Cytochrome C oxidase subunit I of *Rhynocoris marginatus.* The results showed that the *Rhynocoris marginatus* insect gene nucleotide sequence and hydropathy peaks explain the more and less hydrophilic residues and the pair wise alignment and distance were calculated. When compared to other sequences, variations in the nucleotide and amino acid sequence of *R. marginatus* were seen.

Keywords: *Rhynocoris marginatus*, Cytochrome C, Sequence, Sub Unit I. Mitochondrial.

INTRODUCTION

Animal mtDNA typically has a short (15–20 kb) genome with 37 genes. Even while significantly bigger mitochondrial genomes have occasionally been discovered, these are the result of mtDNA duplications rather than changes in gene composition. Assassin bugs have different morphs, biotypes, and ecotypes with various colours and shapes which often mislead a museum entomologist in recognizing the morphs and ecotypes of a particular species (Dunston *et al*., 2014). The assassin bugs of the Rhynocoris species are well known for their role in bio control potential of the insect pests, yet their molecular relationships have not been established at molecular level (Putshko *et al*., 1985, Maldonado, 1990, Baskar *et al*., 2014). The typical insect mitochondrial genome is a circular, double stranded DNA molecule of about 12-20kb in length that contain 37 genes , 13 protein coding genes, 22 transfer RNAs (tRNA) and two ribosomal RNAs (rRNA) Wolstenholme *et al*.,1992; Bore ;1999. Mitochondrial DNA has various interesting properties such as abundance in animal tissue, small size relatively simple genomic structure fast rate of evolution and a straight forward mode of transmission with a low level of recombination (due to its maternal inheritance) This makes it a valuable tool for comparative genomic resolution (Avise *et al*., 1987; Mortz *et al*., 1987 and Arthur Kocher *et al*., 2014). While the genes are dispersed throughout the two strands of certain mtDNAs, all of the genes in other mtDNAs are transcribed from one strand. This is highlighted in the text and in the figures. Positioned in a right-to-left direction. Each strand is transcribed as a single big polycistron in the few situations where it has been studied, and this polycistron is then processed post-transcriptionally to create signals particular to each gene. This study was undertaken based on available mitochondrial sequences of *Rhynocoris marginatus* and we amplified and sequenced the partial COI gene from the mtDNA.

MATERIALS AND METHODS

Collection of *Rhynocoris marginatus*

The adult insect samples for *Rhynocoris marginatus* were gathered in 2018-2019 from the Ayyanar Kovil Tropical Rain forest bordering an agro ecosystem (altitude 389 MSL, latitude 76. 39oE and 10. 45oN) close to Rajapalayam, Virudhunagar District, Tamil Nadu, Southern India. Each type of bug had a minimum of 10

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samples taken, and all the insect specimens were kept in 100% ethanol. After the ethanol had been completely removed from selected samples (n=5), DNA extraction procedures were carried out. Total mtDNA was extracted using the phenol-chloroform method from the thoracic or leg muscles of an individual of the *Rhynocoris marginatus*. The procedure was slightly modified by adding 30 ml of proteinase K (20 mg/ml) and incubating the sample for 16 hours at 52°C.

Polymerase Chain Reaction, sequencing and analysis

Anapproximate of 759bp DNA fragment of the COI gene was amplified for each *Rhynocoris marginatus* by two universal COI gene specific primers: LCO1490F (5' GGTCAACAAATCATAAAGATATTGG-3') and HCO2198R (5'-TAAACTTCAGGGTGA CCAAAAAATCA-3') as reported previously. The PCR products were separated on1.5% agarose gel and visualized by Ethidium bromide (EB) staining. The PCR products were purified using the HiYield PCR/ Gel extraction nkit (RBC Biosciences, Taiwan) following the manufacturer's instructions. The purified amplicons were sequenced using the Big Dye Terminator Cycle sequencing ready reaction kit (Applied Biosystems Inc.,USA) in the ABIprism 3100 Genetic analyzer. The sequencing of COI amplicons of each species (n=5) was performed with the forward and reverse primer, and consensus sequence. Sequenced COI gene of *Rhynocoris marginatus* was assembled and analysed EXPASY translate (Patel *et al*., 1990).

Pairwise sequence alignment

EMBOSS Water uses the Smith- Waterman algoritham to calculate the local alignment of two sequences. Sequence analysis tools services from EMBL-EBI IN 2019. Pair wise distance were carried out with gap opening penalty 15 and gap extension penalty 6.66 (Clustal W) (Thompson *et al*., 1994).

RESULTS AND DISCUSSION

The COI gene of *R. marginatus* has been partially sequence and. Nucleotide sequencing of *R. marginatus* COI gene amplicon disclose an average size of 759 bp (Figure 4). The A+T percentage for the *R. marginatus* COI gene is 29% and G+C percentage is 34.3%. The analysis into divulge the nucleotide frequencies of A-29%, T-37%, C-18% and G-16% (Table 1). Hydropathy plot of the *in-silico* translated amino acid sequence of the *R. marginatus* COI gene protein designates more of hydrophilic residues (mean by the peaks) and less of hydrophobic residues (Figure 1) Molecular weight of the *R. Marginatus* COI gene in 64997.11µ and Residus 1-759, the average residus weight-85.635. Histogram plot of the in silico translated amino acid sequence of the *R.marginatus* COI gene indicates (Figure 2) position from 1to 759 like, Tiny residues and aliphatic, aromatic, non polar, polar residues and positive and negative reidues Pair wise sequence Alignment was carried out with gap and sequence similarity was compared to insect *R. marginatus* COI gene and Cty B gene and the sequence similarity obtained in (37.0%) and COI and COII gene was compared the sequence similarity was observed in (45.6%). The A+T content at each codon position in different insect species have been found to be varial. With the exception *of Apis mellifera* Linnaeus higher A+T values at the third codon position is typical of other insect groups. However, reports from studies desingate more A than T at first codon position and T at second codon position under the order of Hymenoptera (Lunt *et al*., 1996; Navajas *et al*., 1996; Baskar *et al*., 2014; Jeba singh *et al*.,2019) were reported in Rhynocoris insect GC content was 38.7% and AT content was 66.9% and the highest percentage of A+T composition ranging from 61.3 to 66.9% reported in the four species of Rhynocoris insects in Baskar *et al* 2012 and Ambrose *et al* 2014. Dunston Ambrose were reported the genetic diversity of the harpactorine reduviid species of *Rhynocoris marginatus , R.kumarii, R.longifrons* and Livingstone and *R. kolonati* and *R. fusicipes* based on the mitochondrial genes and the present results are correlated with their finding. The present investigation was obtained not only have enriched the knowledge of nucleotide sequence and pair wise comparing the mitochondrial COI and Cyt b and COII (Figure 3) gene its used to analyze the similarities of two genes.

Table 1. Nucleotide composition of the sequenced partial COI gene from the *Rhynocoris marginatus*.

No	Species name	Gene name	Nucleotide sequence obtained (bp)	A	A%	T	T%	C	C%	G	G%	AT%	GC%
1	*R.marginatus*	COI	759	222	29%	277	37%	138	18%	122	16%	66%	34.3%

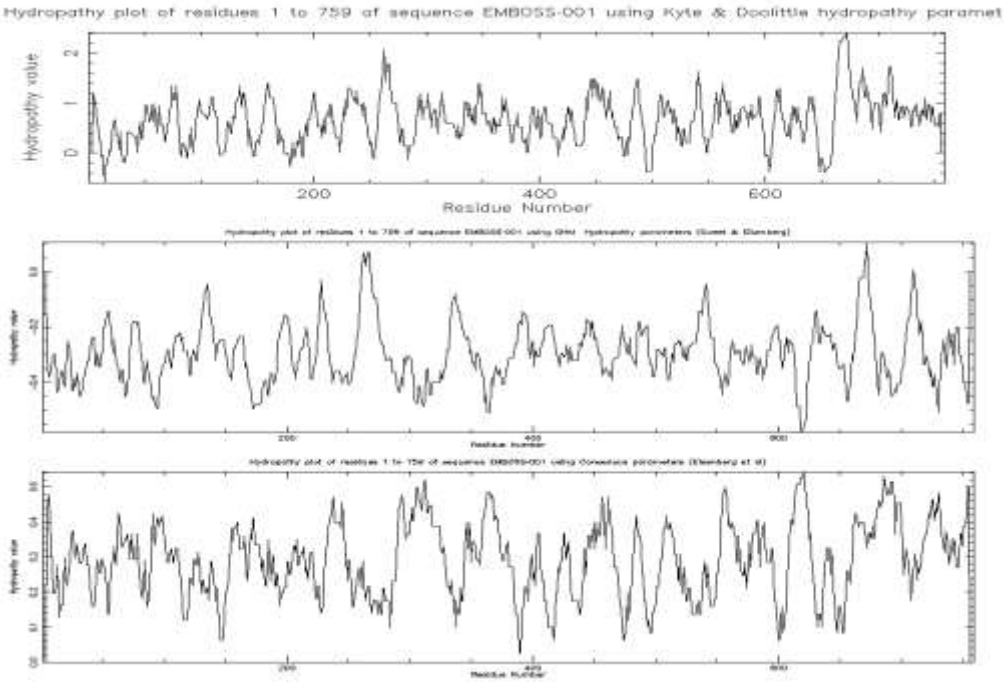

Figure1. Hydropathy plot of the in silico translated partial COI gene protein from the 759 bp nucleotide sequence from *R.marginatus.*

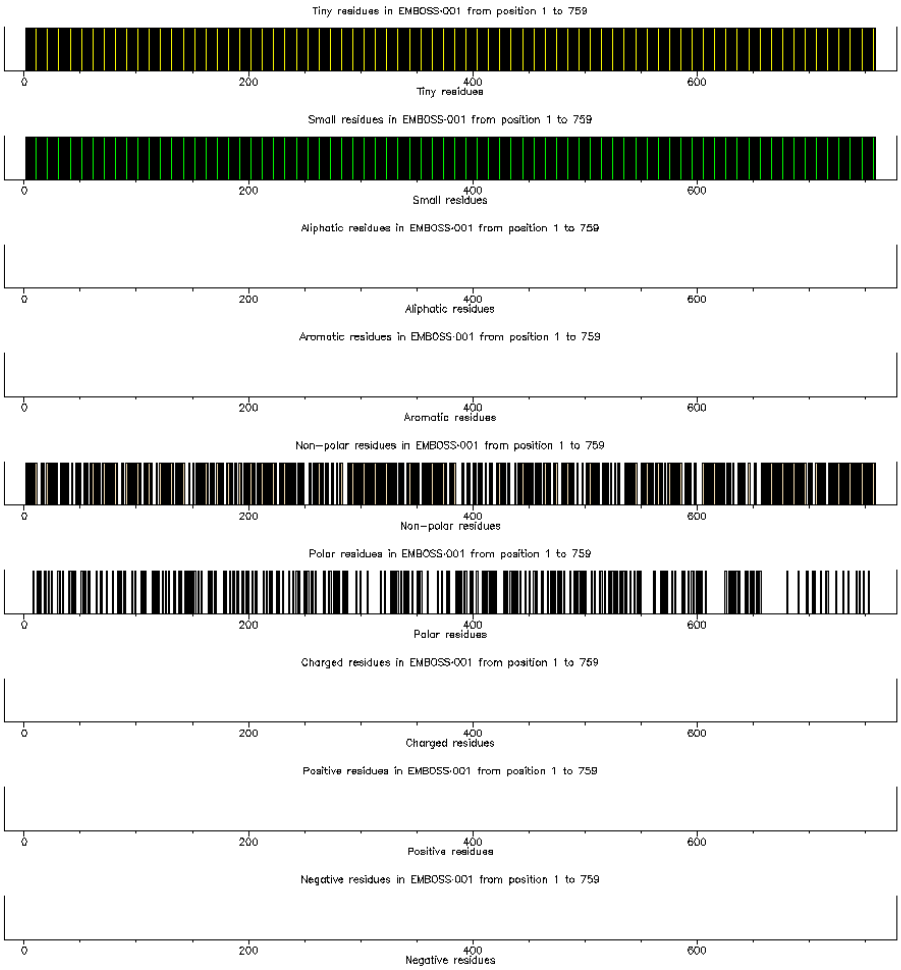

Figure 2. Histogram of sequences.

```
# Program: needle
# Rundate: Fri  9 Jan 2019 12:19:38
# Commandline: needle
#    -auto
#    -stdout
#    -asequence emboss_needle-E20230609-121935-0715-40189646-p2m.asequence
#    -bsequence emboss_needle-E20230609-121935-0715-40189646-p2m.bsequence
#    -gapopen 10.0
#    -gapextend 0.5
#    -endopen 10.0
#    -endextend 0.5
#    -aformat3 pair
#    -sprotein1
#    -sprotein2
# Align_format: pair
# Report_file: stdout
#=======================================
## Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 762
# Identity:     282/762 (37.0%)
# Similarity:   282/762 (37.0%)
# Gaps:         329/762 (43.2%)
# Score: 1006.0
#
#=======================================

EMBOSS_001        1 -------------------------AGAGTAGCATAGGC-AATAGGAG--     22
                                  ||.||.||...|.| ..|..|||
EMBOSS_001        1
CAAGAGCTAATTTTGGGTTCTGATCAGGGTTGCTGGGACTTCTTTGAGAC     50

EMBOSS_001       23 -TATCATTCTGGTTGAAT-------------------------ATGAAC     45
                    |.||||||..|.|||||                         ||||.|
EMBOSS_001       51
TTCTCATTCGAGCTGAATTAGGTACCCCTGGATTTTAATTGGAGATGATC    100

EMBOSS_001       46 TAG--TGTAAC--------TAGTGGTC-TGGCTGGAATGTAATTTTCT--     82
                    .|.  |.||||        ||.||||| ||.||....|.|||||||||
EMBOSS_001      101
AAATTTATAACACTTTTGTTACTGGTCATGCCTTCCCTATAATTTTCTTT    150

EMBOSS_001       83 ---GGATCTCCAA--GTAATTGGGG-------------------------     102
                     |.|...||||  .|||||||.|
EMBOSS_001      151
ATAGTAATACCAATTATAATTGGAGGATTTGGTAATTGATTAGTTCCTCT    200

EMBOSS_001      103 --TTCTCAGAGCG-TTAATAT------------AATAAATATTATCAATG    137
                     |.||..||||. .|.||||           ||||||||.|||.|...
EMBOSS_001      201
AATACTTGGAGCTCCTGATATGGCTTTCCCTCGAATAAATAATATAAGAT    250

EMBOSS_001      138 TT--ACTATTATCCCCATTAAGTCCTTAATTGA----------------     168
                    ||  |.||.|..||||.|....|.|||.|||.|
```

```
EMBOSS_001      251
TTTGATTACTCCCCCCTTCCCTTACTTTATTAATTTTTAGAAGAATCGCT    300

EMBOSS_001      169 -AAAGTAGGGGTGGAA-TGGA--ATC---ATATCCAGATTTCTCTTTAAT
211
                    |||.|.|.|..|||| ||||  |.|   .|||||...|||.||.|.||.
EMBOSS_001      301
GAAAATGGAGCAGGAACTGGATGAACTGTGTATCCTCCTTTATCATCAAA    350

EMBOSS_001      212 -----C-CTAGGGGG------------TTA-----TTTGACC---CTGTT    235
                      | |..||.||           |||     |||..||   |..||
EMBOSS_001      351
TATTGCGCATGGAGGAAATCAGTAGATTTAGCAATTTTTTCCTTACATTT    400

EMBOSS_001      236 TGATG--------TAGGAATAAGAGGTGAATTATTGTTATTGCTGCTAGA
277
                  .|.||       |.....|.||..|. .||||.|.|||||.||.|...|
EMBOSS_001      401 AGCTGCAATTTCTTCTATTTTAGCAGC-
TATTAATTTTATTACTACATCA    449

EMBOSS_001      278 ATAAATGGTA--------GCAGAAAGTATAG----------------TGT    303
                  ||.|||...|       ||...|..|.|.|               |||
EMBOSS_001      450
ATCAATATAAAATTAAATGCTTTATCTTTTGATCAAATACCTTTATTTGT    499

EMBOSS_001      304 AAAGAATCGTGTAAGTATTGCATTATTA--ACTGAGAATCAC--CC----
345
                  ......|..|..||.||.||||||||||  ||| ...|||.|  ||
EMBOSS_001      500 TTGACCTGATAAAATTACTGCATTATTATTACT-
TTTATCCCTACCTGTT    548

EMBOSS_001      346 ----CAGAGTC-----------TTTTTACTAG--GATAT-------CTCC    371
                    |||...|          |.||.|||..  ||.||       ||.|
EMBOSS_001      549
TTAGCAGGAGCAATTACAATATTATTAACTGATCGAAATTTAAATACTTC    598

EMBOSS_001      372 TAGGTAAGGGATTGCTGAGAGGAGG-------------------------    396
                  ....|..|.....||.| |||||||
EMBOSS_001      599 ATTTTTTGATCCAGCAG-
GAGGAGGAGATCCTATTCTTTATCAACATTTA    647

EMBOSS_001      397 ---------TGG------CTCCTC----------------------AGAA    409
                      |||     |.||.|                   ||||
EMBOSS_001      648
TTTTGATTTTGGGGCCCACACCACCCCCCGGGATCAAGACAAATGAAGAA    697

EMBOSS_001      410 T---GATATTCTGTCTC--------CATGGTAAT---CTCC---------    436
                  |  .||.|.|..||.|       |||||.|||   ||||
EMBOSS_001      698
TTAAAATTTCCCATCCCGTTAGCAGCATGGAAATAGGCTCCGGGCTACTA    747

EMBOSS_001      437 ------------    436

EMBOSS_001      748 CTGGAATGAAAG    759
#---------------------------------------
#---------------------------------------
```

Figure 3. DNA Sequence analysis report of pair wise alignment of *Rhynocoris marginatus* COI gene nucleotide sequence.

CONCLUSION

Only a few model animals have had their mitochondrial biology examined, and almost all of the data comes from other insects. Newly discovered mtDNA sequences cast doubt on several ideas that have almost become dogmas. Only a small portion of the Heteroptera: Reduviidae has been thoroughly examined in terms of mtDNA sequences, gene architecture, and molecular mechanisms thus far. Mitochondrial genomics holds considerable promise for unravelling long-forgotten patterns of evolutionary history and acting as a paradigm for genome evolution. In the near future, the comparison of mitochondrial genomic systems may help us better understand numerous patterns of evolution, including those of genomes and organisms.

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